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The Brief Case: An Unusual Cause of Infective Endocarditis after a Urological Procedure

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CASE

A 63-year-old man who underwent transurethral resection of the prostate for benign prostatic hyperplasia presented to his primary care physician for fever and shortness of breath 3 weeks following the procedure. He became apyretic with a moderate improvement of dyspnea after an empirical 7-day course of amoxicillin. Two weeks later, the patient underwent a colonoscopy for rectal bleeding, which revealed only hemorrhoids. One week later, he complained of increasing dyspnea. Cardiac auscultation revealed an aortic diastolic murmur. Transthoracic echocardiography revealed a grade 3 aortic regurgitation and a vegetation of 2 by 2 cm on the tricuspid valve. Subsequently, the patient was admitted to the cardiology ward of the University Hospital of Nancy for infective endocarditis. He underwent tricuspid and aortic valve replacement with bioprosthetic valves because of acute heart failure.

One of the three sets of blood cultures (Becton Dickinson, Le Pont de Claix, France) obtained at admission was positive for Gram-positive bacilli that grew in aerobic and anaerobic bottles after 64 and 37 h of incubation, respectively. After subcultures on tryptic soy agar with 5% sheep blood (TSS) (bioMérieux, Marcy l'Etoile, France) at 37°C in 5% CO_2 atmosphere, isolates were identified by the use of a Vitek mass spectrometry (MS) system (bioMérieux; database version 3.0) as *Actinotignum schaalii* (confidence level of ≥99.9%). A systematic examination of the urine, performed at admission, revealed the presence of 10^5 leukocytes/ml. Urine culture, performed using a nonselective chromogenic agar for nonfastidious uropathogens (UriSelect 4; Bio-Rad, Marnes-la-Coquette, France) and incubated at 37°C aerobically, yielded amoxicillin-susceptible *Escherichia coli* at 10^3 CFU/ml. In order to identify the potential portal of entry of *A. schaalii*, the urine sample obtained prior to antibiotic therapy and stored at 4°C in tubes with preservative (BD Vacutainer with buffered boric acid) for approximately 84 h was reinoculated on selective Columbia colistin and nalidixic acid (CNA) blood agar (bioMérieux) under anaerobic conditions at 37°C. After 48 h of incubation, *A. schaalii* was detected at 10^3 CFU/ml.

Fresh valvular specimens taken during surgery were processed using standard methods and inoculated on Columbia agar with 5% sheep blood (COS), chocolate agar with PolyViteX (PVX), and TSS agar (bioMérieux) as well as into Schaedler broth supplemented with vitamin K (bioMérieux). All media were incubated at 37°C aerobically (Schaedler broth), aerobically with 5% CO_2 (TSS, PVX), or anaerobically (COS). Gross examination of the tricuspid valve specimen confirmed the presence of a vegetation. Rare branched Gram-positive bacilli were seen on Gram stains of ground valvular

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For answers to the self-assessment questions and take-home points, see https://doi.org/10.1128/JCM.01402-17 in this issue.

TABLE 1 Results of antimicrobial susceptibility testing of *A. schaalii*

Antimicrobial agent	MIC (μg/ml)
Penicillin G	0.064
Amoxicillin	0.125
Cefotaxime	0.64
Amikacin	0.75
Gentamicin	0.5
Erythromycin	$<$0.016
Clindamycin	0.016
Tetracycline	0.50
Levofloxacin	0.75
Moxifloxacin	0.25
Vancomycin	0.125
Teicoplanin	0.032
Co-trimoxazole	0.50
Rifampin	0.002
Linezolid	0.75

specimens, while histopathological examination of these tissues demonstrated acute inflammation. Results of broad-range bacterial 16S rRNA gene PCRs performed on fresh specimens of tricuspid and aortic valves were positive. The 658-bp consensus sequence obtained by 16S rRNA gene sequencing of both valves shared 99.1% identity with the sequence of *A. schaalii* CCUG 27420^T, 97.8% with that of *Actinotignum sanguinis* IMMIB L-2199^T, and 93.2% with that of *Actinotignum urinale* CCUG 46093^T (accession numbers FJ960443, HG798952 and AJ439453, respectively). Valve cultures were negative after 7 days of incubation. Results of antibiotic susceptibility testing of the strain isolated from blood cultures are reported in Table 1. Testing was performed using Etest strips (bioMérieux) on Mueller-Hinton agar with 5% sheep blood (bioMérieux). Bacterial suspensions were adjusted to a McFarland standard of 1, and plates were incubated in an anaerobic atmosphere for 48 h, except for aminoglycosides, for which the plates were incubated aerobically with 5% CO_2.

In accordance with the guidelines of the European Society of Cardiology, antibiotic therapy with amoxicillin, cefazolin, and gentamicin was empirically started on the day of surgery (1). Cefazolin therapy was stopped after 5 days, and gentamicin therapy and amoxicillin therapy were continued for a total of 14 and 28 days, respectively, which led to complete clinical and microbiological recovery.

DISCUSSION

Actinotignum (formerly *Actinobaculum*) *schaalii* is a small, Gram-positive, catalase-negative bacillus which may colonize the urogenital tract (2). This facultative anaerobic bacterium is mostly responsible for urinary tract infections (UTIs) in young or elderly patients, particularly in patients with urological diseases, including those who have undergone urological interventions. Abdominal and groin abscesses as well as bacteremia, urosepsis, and spondylodiscitis caused by *A. schaalii* have also been reported (2).

To date, only one case of endocarditis due to *A. schaalii* has been reported (3). That patient, who, in contrast to our case, developed prosthetic valve endocarditis, had been initially hospitalized because of fever and anemia. Gram-positive coryneform rods were identified by Gram staining in blood cultures. While subcultures remained sterile, the patient was given levofloxacin and dismissed without further investigations. That decision may have been related to the fact that coryneforms are frequently considered skin contaminants of blood cultures. Two months later, the patient was again hospitalized with fever. The diagnosis of endocarditis was then established on the basis of clinical and echocardiographic findings and of positive blood cultures yielding *A. schaalii*. In our case, definite native valve endocarditis was diagnosed according to modified Duke criteria (major criteria, positive blood cultures and evidence of endocardial involvement; minor criteria, fever and immunologic phenomena with an increase in the level of rheumatoid factor) (1). Moreover, *A. schaalii* was detected by pan-bacterial PCR in the two valves, which confirms its involvement.

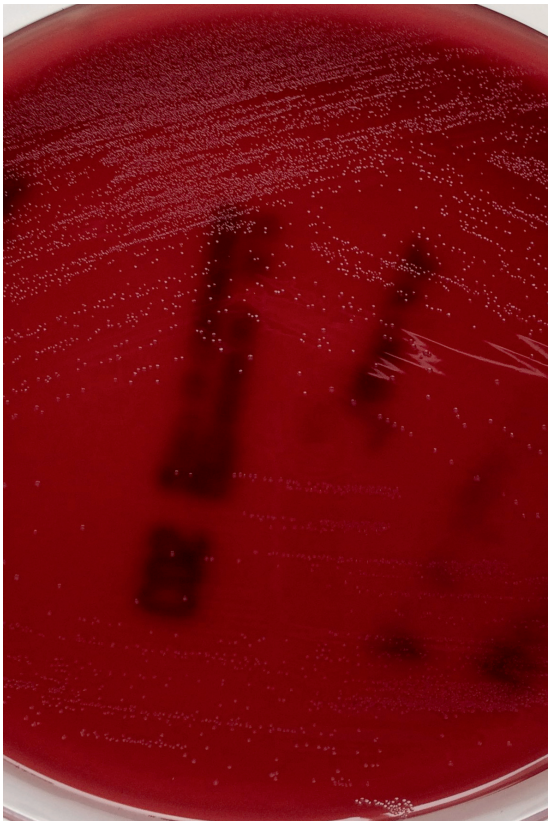

FIG 1 Colonies of *A. schaalii* on blood agar after 48 h of incubation under anaerobic conditions.

Dissemination of infections by *A. schaalii* is most likely a result of urinary tract translocations. However, no portal of entry could be identified in the previously reported case of *A. schaalii* endocarditis (3), which involved a patient without evidence of underlying urological abnormalities or signs of UTI, while urine culture was reported as sterile. In our case, resection of the prostate could have been the origin of *A. schaalii* bacteremia, as it was followed by an episode of fever and dyspnea treated briefly by amoxicillin, which might have delayed the clinical picture of endocarditis. Initial urine examination results in our patient were consistent with an UTI due to *E. coli*. However, *A. schaalii* was recovered at 10^3 CFU/ml after reinoculation of the urine sample on selective CNA agar incubated anaerobically. These findings support the hypothesis that the origin of the infection might have been the urogenital tract. Considering the worsening of the patient's clinical condition after colonoscopy, a digestive portal of entry was considered. However, this hypothesis remains very unlikely, considering that *A. schaalii* has not been reported to be a part of the intestinal microbiota (2).

A. schaalii grows slowly (≥48 h) on blood-enriched media under conditions of 5% CO_2 or of an anaerobic atmosphere. Colonies are tiny, gray, and nonhemolytic or weakly beta-hemolytic after 3 to 5 days of incubation (Fig. 1) and can be distinguished from colonies of nonhemolytic Gram-positive cocci by Gram staining (4). The detection of *A. schaalii* in urine may be difficult since in many laboratories a standard urine culture does not include blood-enriched media incubated anaerobically or under conditions of 5% CO_2 for 48 to 72 h and because urine samples are often not examined by microscopy after Gram staining.

Microbiologists should always consider the possibility of the presence of *A. schaalii* infection in young or elderly patients with leukocyturia when standard chromogenic media remain sterile after 24 h of incubation. In such cases, blood agar plates should be inoculated and incubated at 37°C under 5% CO_2 conditions and/or anaerobically for 48 h. Blood agar should always be used for urine culture in the case of a positive direct

examination revealing small coccoid Gram-positive rods and also, if clinical data are available, in cases of unexplained recurrent UTIs, in young or elderly patients with an underlying disease, and of UTIs not responding to trimethoprim, co-trimoxazole, or ciprofloxacin (2). In laboratories where flow cytometry is available, a cost-effective algorithmic approach can be used, as proposed by Lotte et al. (5). Urine samples are first analyzed by a flow cytometer (UF1000i; bioMérieux). When the urinary leukocyte count is >50/μl and the bacterial count is >14/μl and/or the number of small particles (particles that do not correspond to any well-defined parameter but that may correspond to small bacteria such as *A. schaalii*) is >7,500/μl, urine is seeded on blood agar.

The diagnosis of *A. schaalii* infection may be further impaired by identification problems. API system tests do not include this species in their database and may misidentify it as *Gardnerella vaginalis*, *Arcanobacterium* spp., *Actinomyces meyeri*, or *Actinomyces israelii* (2). In contrast, satisfactory identification rates have been reported with the Vitek 2 ANC card (bioMérieux) (2). Molecular methods such as 16S rRNA gene sequencing and specific real-time PCR are also reliable but are costly and not readily available. The emerging fast and inexpensive matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) identification method is a promising tool for accurate identification of *A. schaalii*. We recently compared two commercially available *in vitro* diagnostic (IVD) systems, MALDI Biotyper (Bruker Daltonics, Wissembourg, France; 5,627 database entries) and Vitek MS (bioMérieux; database version 2.0) for the identification of *Actinomycetaceae*, including *A. schaalii* (using 16S rRNA gene sequencing identification as a gold standard) (6). In our study, MALDI Biotyper identified 11 of 20 *A. schaalii* strains tested using direct deposit (score of ≥2.0). All 20 isolates have been accurately identified after on-plate formic acid treatment. *A. schaalii* was not included in the Vitek MS 2.0 database, leading to an absence of identification for 17 strains and 3 misidentifications as *Micrococcus luteus*, *Streptococcus agalactiae*, and *Kytococcus sedentarius* (confidence level of ≥90%). However, testing with the more recent Vitek MS 3.0 database allowed a reliable identification of 18 of the 19 tested isolates after direct deposit (confidence level of ≥99.9%).

A. schaalii is usually susceptible to all β-lactams (except amdinocillin), tetracyclines, glycopeptides, linezolid, rifampin, aminoglycosides, and nitrofurantoin. Some strains may be resistant to macrolides and clindamycin (2). *A. schaalii* is frequently reported to be resistant to co-trimoxazole and quinolones (norfloxacin and ciprofloxacin) (2). Resistance to these antibiotics, widely used in the treatment of UTIs, is problematic. Resistance to fluoroquinolones or co-trimoxazole is also a matter of concern with regard to antibiotic prophylaxis for urological procedure since these antibiotics may be used as first-choice drugs in this indication. Although there are no specific treatment guidelines, it seems that the treatment of *A. schaalii*-related infections should be continued for up to 2 weeks or more, depending on the infection site (2). In our patient, 2 weeks of gentamicin therapy and 28 days of amoxicillin therapy resulted in complete clinical and microbiological recovery.

A. schaalii is recognized as an emerging uropathogen. Our case highlights the fact that this organism can also be responsible for serious systemic infections. Improved detection in urine samples using blood agar incubation under conditions of 5% CO_2, and reliable identification with MALDI-TOF mass spectrometry, will help to better estimate its real prevalence and pathogenicity.

SELF-ASSESSMENT QUESTIONS

1. What is the usual habitat of *Actinotignum schaalii*?
    A. The digestive tract
    B. The oropharynx
    C. The skin
    D. The genitourinary tract
2. *Actinotignum schaalii* grows easily under what conditions?
    A. On MacConkey agar under aerobic conditions

B. On MacConkey agar under conditions of a 5% CO_2 atmosphere
C. On Trypticase soy agar with 5% sheep blood under aerobic conditions
D. On Trypticase soy agar with 5% sheep blood under conditions of a 5% CO_2 atmosphere

3. *Actinotignum schaalii* is usually susceptible to what antibiotic?

A. Amoxicillin
B. Co-trimoxazole
C. Ciprofloxacin
D. Amdinocillin

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